

*Ex 8
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C-16* 21. (twice amended) The polynucleotide of Claim 16, wherein the polynucleotide comprises SEQ ID NO: 13.

Remarks

Applicant thanks the Examiner for the courtesies extended in the telephone conversation of October 2, 2002. Applicant provides the instant Supplemental Response to Restriction Requirement per the Examiner's suggestion.

In the instant Supplemental Response, Claims 16, 20, and 21 are amended and the paragraphs indicated above have been modified to conform USPTO policy or to correct minor clerical errors. As seen in the remarks below, no new matter is believed to be at issue.

Claims 16, 20, and 21 have been amended to be drawn to a polynucleotide encoding the polypeptide of SEQ ID NO:14.

The paragraphs starting on Page 6 at Line 36 and Page 18 at Line 3 have been amended to remove hyperlinks to the world wide web which is the preference of the USPTO. The paragraph on Page 2 at Line 19 was amended to read "a nucleic acid fragment encoding an acid or a neutral acid triacylglycerol lipase." Support for this amendment is found on page 2 at line 22. The paragraph starting on Page 2 at Line 33 was amended to agree with the Figure 1.

The paragraph starting on Page 23 was amended at lines 7, 12, and 17. At line 12 "SEQ ID NO:23" was replaced by "SEQ ID NO:24," support for this amendment is found in page 4 at lines 17-22. At line 7 "SEQ ID NO:2" was replaced with "SEQ ID NO:22" and at line 17 "SEQ ID NO:4" was replaced by "SEQ ID NO:26". These last two identification numbers were replaced because, as stated in Table 5 on page 21, SEQ ID NO:2 and SEQ ID NO:4 encode polypeptides homologous to *C. elegans* and *C. familiaris* acid triacylglycerol lipases. As stated in Table 8 on page 24, SEQ ID NO:22 and SEQ ID NO:26 encode polypeptides homologous to *C. elegans*, *R. miehei*, and *T. lanuginosus* neutral triacylglycerol lipases.

SEQ ID NO: 36 was removed from the last row of Table 8 was removed because SEQ ID NO:36 is described in the specification as filed, page 5, lines 12-13, as "the amino acid sequence of a *Caenorhabditis elegans* acid triacylglycerol lipase, NCBI General Identifier No. 3165581." Clone wr1.pk0115.f5 is from wheat, not *Caenorhabditis elegans*. See Table 2, last entry, page 17.

In view of the foregoing, allowance of the application is earnestly solicited.

Respectfully submitted,

Lori Y. Beadell
LORI Y. BEARDELL
Attorney For Applicants
Registration No. 34,293
Telephone: 302-992-4926
Facsimile: 302-892-1026

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In showing the changes, deleted material is shown within brackets, and inserted material is shown underlined.

IN THE SPECIFICATION:

Please replace the following paragraphs:

Paragraph starting on Page 2 at Line 19:

An additional embodiment of the instant invention concerns a method of altering the level of expression of an acid or a neutral triacylglycerol lipase in a transformed host cell comprising: a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding an acid or a neutral acid triacylglycerol lipase; and b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of acid or neutral triacylglycerol lipase in the transformed host cell.

Paragraph starting on Page 2 at Line 33:

Figure 1A-C depicts the amino acid sequence alignment between the acid triacylglycerol lipase from rice clone rlr72.pk0015.b2 (SEQ ID NO:14), soybean contig assembled from clones sdp3c.pk004.n3 and ssl.pk0022.a1 (SEQ ID NO:18), soybean contig assembled from clones sls1c.pk009.o2, srr1c.pk001.m19 and sre.pk0004.d7 (SEQ ID NO:20), *Canis familiaris* (NCBI General Identifier No. 3041702, SEQ ID NO:35) and *Caenorhabditis elegans* (NCBI General Identifier No. 3165581, SEQ ID NO:36). Amino acids which are conserved among all sequences are indicated with an asterisk (*) while amino acids conserved only among plant sequences are indicated by a plus sign (+). Dashes are used by the program to maximize alignment of the sequences. Figure 1A, amino acids 1-180, Figure 1B, amino acids 181-360, and Figure 1C, amino acids 361-433.

Paragraph starting on Page 6 at Line 36:

A "substantial portion" of an amino acid or nucleotide sequence comprises enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to afford putative identification of that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al. (1993) *J. Mol. Biol.* 215:403-410[; see also www.ncbi.nlm.nih.gov/BLAST/]). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage

plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches partial or complete amino acid and nucleotide sequences encoding one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

Paragraph starting on Page 18 at Line 3:

ESTs encoding triacylglycerol lipases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al. (1993) *J. Mol. Biol.* 215:403-410[; see also www.ncbi.nlm.nih.gov/BLAST/]) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish, W. and States, D. J. (1993) *Nature Genetics* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

Paragraph starting on Page 23 at Line 1:

The sequence of the entire cDNA insert in clone cr1n.pk0145.c6 was determined and is shown in SEQ ID NO:21; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:22. The amino acid sequence set forth in SEQ ID NO:[2] 22 was evaluated by BLASTP, yielding a pLog value of 10.70 versus the *C. elegans* sequence. The sequence of the contig assembled from a portion of the cDNA insert in clones p0010.cbpbe40r, p0083.cldcq17r, p0048.cqlac25r, p0118.chsbw59r, cr1.pk0011.c9 and cdo1c.pk002.c22 is shown in SEQ ID NO:23; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:24[23]. The sequence of the entire cDNA insert in clone cr1n.pk0127.h8 was

determined and a contig assembled with this sequence and the sequence from a portion of the cDNA insert in clones p0037.crwan02r, p0004.cb1fm22r, p0004.cb1ei43r, cco1n.pk068.o9 and p0093.cssao39r. The sequence of this contig is shown in SEQ ID NO:25; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:26. The amino acid sequence set forth in SEQ ID NO:26[4] was evaluated by BLASTP, yielding a pLog value of 9.70 versus the *Thermomyces lanuginosus* sequence. The sequence of a portion of the cDNA insert from clone rdr1f.pk002.f11 is shown in SEQ ID NO:27; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:28. The sequence of the entire cDNA insert in clone sre.pk0058.b1 was determined and a contig assembled with this sequence and the sequence of a portion of the cDNA insert in clone sah1c.pk001.k20. The sequence of this contig is shown in SEQ ID NO:29; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:30. The amino acid sequence set forth in SEQ ID NO:30 was evaluated by BLASTP, yielding a pLog value of 8.05 versus the *Rhizomucor miehei* sequence. The sequence of the entire cDNA insert in clone sr1.pk0079.e1 was determined and is shown in SEQ ID NO:31; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:32. The amino acid sequence set forth in SEQ ID NO:32 was evaluated by BLASTP, yielding a pLog value of 7.52 versus the *Rhizopus niveus* sequence. The sequence of the entire cDNA insert in clone wr1.pk0115.f5 was determined and is shown in SEQ ID NO:33; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:34. The amino acid sequence set forth in SEQ ID NO:34 was evaluated by BLASTP, yielding a pLog value of 13.52 versus the *Caenorhabditis elegans* sequence.

Please replace the following table:

TABLE 8

Percent Similarity of Amino Acid Sequences Deduced From the Nucleotide Sequences
of cDNA Clones Encoding Polypeptides Homologous
to Neutral Triacylglycerol Lipase

Clone	SEQ ID NO.	Percent Similarity to		
		3877256	2997733	417256
cr1n.pk0145.c6	22	15.1	13.2	16.8
Contig of:	24	60.5	17.5	22.9
p0010.cbpbe40r				
p0083.cldcq17r				
p0048.cqlac25r				
p0118.chsbw59r				
cr1.pk0011.c9				
cdolc.pk002.c22				
Contig of:	26	18.5	14.3	15.1
p0037.crwan02r				
p0004.cb1fm22r				
p0004.cb1ei43r				
cco1n.pk068.o9				
p0093.cssao39r				
cr1n.pk0127.h8				
rdr1f.pk002.f11	28	12.6	20.6	22.9
Contig of:	32	15.1	10.5	17.0
sah1c.pk001.k20				
sre.pk0058.b1				
sr1.pk0079.e1	34	14.3	21.1	24.6
wr1.pk0115.f5	[36]	37.0	22.0	26.0

IN THE CLAIMS:

Please amend claims 16, 20, and 21 as follows:

16. (twice amended) An isolated polynucleotide comprising: (a) a nucleotide sequence encoding a polypeptide having triacylglycerol lipase activity, wherein the polypeptide has an amino acid sequence of at least 80% sequence identity, based on the Clustal method of alignment, when compared to SEQ ID NO:[12]14; or (b) a complement of the nucleotide sequence, wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary.

20. (twice amended) The polynucleotide of Claim 16 wherein the amino acid sequence of the polypeptide comprises SEQ ID NO:[12]14.

21. (twice amended) The polynucleotide of Claim 16, wherein the polynucleotide comprises SEQ ID NO:[11]13.